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Magnetically Tagged Subsets of Human Lymphocytes for Assays with Laboratory Robotics

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We demonstrate the possibility of automation of whole-cell functionality assays, e.g., mitogen-activated DNA synthesis, DNA repair synthesis, and assessment of drug-metabolizing enzymes, by use of magnetic separation technology. We have attached antibody-coupled magnetic microspheres to the surface of human T-lymphocytes before performing various assays. Evaluating the biological functions of T-cells estimated by the DNA-synthesis assays showed that the presence of antibody-coupled magnetic microspheres did not affect the results ($P > 0.05$). The concentration of adenosine diphosphate ribosyltransferase (EC 2.4.2.30) was shown to be influenced by the magnetic microspheres. However, the amount of enzyme activity induced by oxidative stress was not significantly altered. The results from assays of the phase II drug-metabolizing enzymes glutathione transferase (EC 2.5.1.18) and epoxide hydrolase (EC 3.3.2.3) as well as evaluation of the proliferative response of polyclonal activators (phytohemagglutinin, staphylococcal enterotoxin A, and pokeweed mitogen) support our conclusion that assays can be performed on viable magnetized cells. The use of magnetized cells holds promise for further applications in automated genotoxic and immunological cell assays of mononuclear leukocyte subsets. Laboratory robotics will be essential in bringing these assays into routine use.

Additional Keyphrases: *cell viability studies · DNA synthesis · drug-metabolizing enzymes · mononuclear leukocytes · use of magnetizable cell separations*

Whole-cell functionality assays are at present not regarded as tools within clinical chemistry. The procedures currently used for these assays are time consuming and tedious to perform, mainly because of the separation technology (centrifugation) now in use. A normal centrifugation step loses about 10–20% of the cells, so that large blood-sample volumes, i.e., >30 mL, must be drawn, which makes the assays less suitable for human use. However, with the use of magnetic tagging and separation, a higher yield can be achieved in each step, so that "normal" blood-sample volumes from patients can be used.

Many chemicals to which humans are exposed have not been properly tested for carcinogenicity because of the lack of appropriate tests at a reasonable cost. At present, the major regulatory decisions are based on results of long-term animal testing. However, Lave and Omenn (*1*) have argued that these assays can be performed as in vitro short-term tests. Indeed, the assay procedures we describe here have already been utilized in manual assessment of genotoxic exposures. However, most of the tests Lave and Omenn discussed (*1*) involved animal cells or immortalized human cell lines, both of which present difficulties for extrapolating the results to humans.

Here we present an assay system based on the use of magnetically tagged human lymphocytes, to shorten assay time and to provide a means of automation, so that the assays can be reproducibly performed with higher throughput and lower cost than in manual procedures. Furthermore, automation of cell assays gives us an opportunity to perform assays on cells that until now have been regarded as too time consuming and expensive for use in clinical laboratories, e.g., assessment of drug-metabolizing enzymes or of DNA repair synthesis.

Materials and Methods

Materials

Antibodies. We used the following monoclonal antibodies (mAbs): Mark-1, mouse anti-rat kappa light chain hybridoma obtained from H. Bazin (*2*); RAMOL-1, rat anti-mouse Ig light chains, a gift from Dr. Brodin (*3*); anti-CD8 and anti-CD4 hybridomas, obtained from the American Culture Collection (NIH, Bethesda, MD); and anti-CD2 obtained from Coulter Corporation (Hialeah, FL).[5]

Cells. The cells studied were peripheral blood mononuclear cells (MNC) from human donors. We diluted the blood fivefold with heparinized phosphate-buffered saline (per liter, 20 mmol of phosphate, 0.154 mmol of NaCl, pH 7.4, and 25 000 int. units of heparin) and separated them on a Ficoll-Paque cushion (density 1.077 kg/L) by centrifugation ($400 \times g$ for 30 min). The interface cells were washed three times in RPMI-1640 medium (Flow Laboratories, Solna, Sweden) supplemented with, per liter, 10 mmol of 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 2 mmol of L-glutamine, and 10 g of bovine serum albumin, (R1BSA-solution). Residual erythrocytes were lysed by hypotonic shock. The MNC were counted in a hemocytometer and resuspended in R1BSA. MNC viability, determined by trypan blue exclusion, exceeded 98%.

T lymphocytes were isolated from the MNC by the addition of sheep erythrocytes, activated with *S*-2-aminoethylisothiouronium hydrobromide ("AET"; Sigma Chemical Co., St. Louis, MO), which formed rosettes with the T cells (*4*).

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[5] Nonstandard abbreviations: mAb, monoclonal antibody; MNC, mononuclear cells; R1BSA, RMPI-1640 medium supplemented with L-glutamine and bovine serum albumin in HEPES buffer (see text); ADPRT, adenosine diphosphate ribosyltransferase; UDS, unscheduled DNA synthesis; GT, glutathione transferase; NA-AAF, *N*-acetoxy-2-acetylaminofluorene; and tSBO, *trans*-stilbene oxide.

Procedures

Magnetic microsphere production. We prepared the magnetic microspheres and subsequently attached mAbs to their surface as described previously (*5*). In brief, we dissolved 500 mg of starch in 1.6 mL of water, added 300 μL of a magnetite suspension ("EMG 805 HMGS"; Ferrofluid Corp., Nashua, NH), and emulsified this in vegetable oil by pumping it through a high-pressure homogenizer. To stabilize the microspheres, we slowly poured the emulsion into acetone while stirring. After washing the magnetic microspheres with acetone, we activated the particles by adding 2,2,2-trifluoroethanesulfonyl chloride (*6*). The mAbs, at a concentration of 1.3–2.0 g/L, were covalently coupled at room temperature for 1 h to the activated magnetic microspheres in carbonate buffer (0.1 mol/L, pH 9.6), yielding about 150 μg of mAb per milligram of magnetic microspheres (dry weight).

Magnetic separation. mAb-coupled magnetic microspheres were suspended and added to MNC. The mixture was incubated under mild agitation for 1 h at 4 °C in RIBSA. To magnetically separate the MNC–magnetic microsphere suspension, we used cobalt/samarium permanent magnets on the outside of the test tube. Cells with attached magnetic microspheres were retained by the magnet and used in the subsequent assay. Carried out at 4 °C, the magnetic separation procedure was completed within about 15 min. To determine the relative number of cells identified by the various mAbs, we used an Epics C flow cytofluorometer (Coulter Electronics, Hialeah, FL) and fluorescein-labeled mAbs. After the magnetic separation we determined cell recovery and viability of the fractions by counting the cells in a hemocytometer.

Robotics. We programmed a laboratory robot system (robot arm, software, and dispensing equipment from Perkin-Elmer, Palo Alto, CA) to perform the cell separation, starting with Ficoll-separated MNC, and the adenosine diphosphate ribosyltransferase (ADPRT; EC 2.4.2.30) assay in exactly the same way as done manually. Only the actual adding of scintillation fluids and the measuring with the beta-counter were performed manually.

Interfacing, error handling, and programming of the robot system were performed according to the manufacturer. All other items used in the robotic set-up were standard laboratory equipment.

For comparing the manual and the robotic procedures, we used the robot to perform the cell separation, then divided the sample for analysis by a manual and a robotic assay procedure.

Analysis of Biochemical Viability of Cells

Different cell populations will give different results in the same genotoxic assay (*7–10*). Thus, to determine whether antibodies or magnetic microspheres attached to the surface of the cells would alter the final results of the different assays, we chose to perform the assays with lymphocytes, which could be separated by rosetting, taking advantage of their "sheep erythrocyte" receptor, a receptor that is also identified by the mAb anti-CD2. In this way, we could use for comparison a closely defined cell population that had not been contacted with any mAbs. In the cell separation involving magnetic microspheres, we also used a combination of anti-CD4 and anti-CD8 antibodies. These two mAbs together define almost 100% of the rosetted T-cell population, as does anti-CD2. Using sheep erythrocytes and th anti-CD2 mAb, we could perform the assay on the same subpopulation of cells, with or without antibody or magnetic microspheres attached to the surfaces of the target cells.

Unscheduled DNA synthesis (UDS), induced with N-acetoxy-2-acetylaminofluorene (NA-AAF), and NA-AAF binding to DNA. The details of these procedures have been published lsewhere (*11*). Briefly, for NA-AAF binding to DNA, we cultured T lymphocytes, with or without antibodies or antibody-coupled magnetic microspheres, in Hank's Eagle medium (Flow Laboratories) fortified with human platelet-poor plasma, 10 mL/L, then exposed them to 1 μmol of [^{3}H]NA-AAF (5.18 kCi/mol; Midwest Research Institute, Kansas City, MO) for 30 min at 37 °C. After exposure, the cells were harvested by centrifugation at 400 × *g*. The NA-AAF binding to DNA was determined by extraction and purification of the cellular DNA, followed by quantification of the bound radioactivity (counts/min of [^{3}H]NA-AAF per microgram of DNA).

Cells were also exposed to 10 μmol of unlabeled NA-AAF in Hank's Eagle medium supplemented with human platelet-poor plasma, 200 mL/L (obtained by centrifuging platelet-rich plasma for 10 min at 600 × *g*). After 30 min at 37 °C the culture medium was replaced by fresh medium of the same kind and further incubated for 18 h at 37 °C in the presence of hydroxyurea, 10 mmol/L, and [^{3}H]Thd (25 kCi/mol; Amersham Int., Bucks., U.K.), 10 mCi/L. The UDS could then be estimated as counts/min of [^{3}H]Thd incorporated per microgram of DNA, after subtracting the counts/min of [^{3}H]Thd incorporated per microgram of DNA in a control culture that had not received NA-AAF. The variability of our methods carried out as described here was <19.3% for eight separate experiments repeated at weekly intervals on cells from the same individuals (*12*).

Drug-metabolizing enzymatic assays. We determined the glutathione transferase (GT; EC 2.5.1.18) activity toward [^{3}H]*trans*-stilbene oxide (tSBO) by a procedure already reported (*13*). In brief, T lymphocytes, with or without antibodies or antibody-coupled magnetic microspheres, were incubated with and without reduced glutathione, 5 mmol/L, at 37 °C. Eight minutes after adding 250 μmol of [^{3}H]tSBO, we terminated the reaction by adding hexanol, shook vigorously, then centrifugated the sample in a desktop centrifuge to obtain phase separation. Radioactivity in the aliquots from the lower (aqueous) phase, where the glutathione-conjugated product remained, was counted by liquid scintillation. We expressed the activity as picomoles of conjugate per minute per 10^7 cells.

We also determined activities of soluble and membrane-bound epoxide hydrolase (EC 3.3.2.3) towards [^{3}H]tSBO and [^{3}H]*cis*-stilbene oxide, respectively (*14*). Briefly, we incubated T lymphocytes for 60 min at 37 °C in isotonic saline, with or without antibodies or antibody-coupled magnetic microspheres. Adding the substrates ([^{3}H]tSBO, 25 μmol, or [^{3}H]*cis*-stilbene oxide, 60 μmol) started the reactions. We terminated the reactions by adding dodecane, shook vigorously, then centrifugated for phase separation. Radioactivity in the aqueous phase was counted as above and expressed as picomoles of product formed per minute per 10^7 cells.

Adenosine diphosphate ribosyltransferase activity. We determined the activity of ADPRT by a procedure reported earlier (*15*). In brief, we incubated T lymphocytes, with or without antibodies or antibody-coupled magnetic microspheres, with H_2O_2 for 15 min. We removed the peroxide by magnetic washing and permeabilized the plasma mem-

branes at 4 °C for 15 min with a Tris · HCl buffer (Tris, 10 mmol/L at pH 7.8; EDTA, 1 mmol/L; 2-mercaptoethanol, 30 mmol/L; and $MgCl_2$, 4 mmol/L). After incubation for 15 min with [^{3}H]NAD^+, we stopped the reaction by adding NaCl, 3.7 mol/L. We recorded the data as counts/min of [^{3}H]NAD^+ incorporated into trichloroacetic acid-precipitable material per microgram of DNA.

T-cell stimulation tests. By a passage over a gelatin column, we partly depleted human MNC of monocytes as described (*16*), then incubated the remaining cells with a mixture of mAbs OKT4 and OKT8. We then added magnetic microspheres to which RAMOL-1 had been coupled and magnetically separated the cells as described above. By incubating a sample of the cells overnight at 37 °C, the great majority of the cells was entirely free of particles, owing to extensive release of microspheres. However, a few cells with a very low concentration of particles still remained. We then tested cells not exposed to microspheres, cells with attached microspheres, and cells after detachment of microspheres for thoir proliferative response to the polyclonal activators phytohemagglutinin, staphylococcal enterotoxin A, or pokeweed mitogen. After 72 h, we also routinely determined, in triplicate, the DNA synthesis by [^{3}H]thymidine incorporation as described (*17*).

Results

In all the experiments reported below we isolated the T lymphocytes from blood donors so as to make them at least 98% positive for the anti-CD2 antibody. Table 1 shows the effect of the magnetized anti-CD2-coupled antibodies at the surface of T-lymphocytes on UDS, on NA-AAF binding to DNA, and on hydroxyurea-suppressed DNA synthesis. We observed no significant effect by the bound antibodies and particles on the assay results. We conclude that the enzyme systems involved in the quantification are not affected by the presence of antibody-coupled magnetic microspheres.

UDS is influenced by the DNA repair enzymes (i.e., endonuclease, exonuclease, polymerase, ligase). Likewise, UDS and DNA synthesis are influenced by deoxynucleotide pools and their precursor metabolism. NA-AAF binding is influenced by esterases that possess deacetylase activity. Therefore, we found through extrapolation that none of these biological functions seem to be profoundly affected in T lymphocytes that carry magnetized anti-CD2-coupled particles.

Reduced iron (Fe^{3+}) is well known to catalyze oxygen radical production via the Fenton reaction in biological systems (*18*). ADPRT, a nucleotide-associated enzyme involved in DNA repair, cell proliferation, differentiation, and gene expression, is also activated by DNA strand breaks, including those introduced by oxidative stress (*19*). As Table 2 indicates, the ADPRT activity is influenced by the presence of magnetized iron particles, but the amount of ADPRT activity induced by the standardized 100-μmol dose of H_2O_2 was not significantly altered.

Table 3 illustrates the influence of the presence of antibody-coupled magnetic microspheres at the lymphocyte surface on the quantitative estimation of phase II drug-metabolizing enzymes: GT and epoxide hydrolase. These enzymes are influenced by the cellular presence of oxides and epoxides often produced by the mixed-function oxidases, some of which may be genotoxic and related to iron-influenced production of oxygen radicals.

Table 3 shows that GT-tSBO activity can best be measured in magnetically tagged T-lymphocytes in the presence of glutathione (5 mmol/L), a co-substrate for GT-tSBO activity. It did not help the GT-tSBO assay that we permeabilized the cells by sonication. On the contrary, estimations of soluble epoxide hydrolase activity substantially improved when we sonicated the magnetized cells before performing the assay. On the other hand, membrane-bound epoxide hydrolase was unaffected by the presence of antibody-coupled magnetic microspheres when we performed the assay in isotonic saline. The coefficients of variation for these assays are between 9% and 11% (*13, 14*). Together, these data support our conclusion that valid assays for GT or epoxide hydrolase can be performed on cells that carry antibody-coupled magnetic microspheres, because the cells remain viable.

Table 4 summarizes results for assays of ADPRT performed by the robot and by the manual procedure. Differences between manual and robotic procedures for the effect of peroxide stimulation are well within the error of the assay. This demonstrates the reproducibility of the results obtained for cells having surface-attached particles even when subjected to the robotic procedure.

We also evaluated the proliferative responsiveness of MNC to polyclonal activators with attached magnetic microspheres as compared with the response of samples from the same cell population but without exposure to microspheres or after detachment of the microspheres. The presence of microspheres on the cells did not preclude a proliferative cell response, although the amplitude of the response was sometimes significantly reduced (Table 5). The response tended to increase somewhat from those lowest values, when cells were tested after the micro-

Table 1. Effect of Antibody-Coupled Magnetic Microspheres on the Biological Responsiveness of Human T Lymphocytes

	Hydroxyurea-suppressed DNA synthesis	NA-AAF-induced UDS	NA-AAF binding to DNA
	Counts/min per μg of DNA (mean ± SEM)		
With antibodies	105 ± 5	241 ± 5	100 ± 18
Without antibodies	114 ± 7	255 ± 12	125 ± 7
With magnetic particles	122 ± 4	249 ± 13	57 ± 2
Without magnetic particles	112 ± 5	251 ± 12	64 ± 4

n = 4 each. All differences are nonsignificant ($P > 0.05$) by Student's *t*-test.

Table 2. Effect of Antibody-Coupled Magnetic Microspheres Adhering to Human T Lymphocytes on ADPRT Activity

Type of cell[a]	[^{3}H]NAD^+, counts/min incorporated per μg of DNA: Mean	SEM	Mean difference
L1	228	21	
L1 + H_2O_2	1167	217	939
M^+	693	53	
M^+ + H_2O_2	1546	203	802

[a] L1 = T cells separated by rosetting with sheep erythrocytes (n = 2 each), M^+ = $CD4^+$ and $CD8^+$ T cells positively separated with magnetic particles (n = 4 each).

Table 3. Effect of Antibody-Coupled Magnetic Microspheres on Enzyme Activity of Human T Lymphocytes

	T lymphocytes only			T lymphocytes attached to mAb CD8 antibody-coupled magnetic microspheres		
	Specific acty[a]	% of control	Background, pmol[b]	Specific acty[a]	% of control	Background, pmol[b]
Glutathione transferase activity (n = 4)						
In saline (NaCl, 8.5 g/L)	196	100		78	40	
In saline + GSH, 5 mmol/L	480	100		262	55	
Sonicated in saline + GSH	1396	100		508	36	
Soluble epoxide hydrolase activity (n = 2)						
In saline	11.1	100	402	4.2	38	59
Sonicated in saline	12.0	100	38	7.8	65	12
Membrane-bound epoxide hydrolase activity (n = 2)						
In saline	8.8	100		6.8	77	
In saline	10.5	100		9.9	94	
Sonicated in saline	10.1	100		5.7	56	

[a] Picomoles of product formed per minute per 10^7 cells. [b] Nonenzymatic conjugation. GSH, glutathione.

Table 4. Robotic Cell Separation and Assay of ADPRT[a]

	ADPRT acty, counts/min ([^{3}H]NAD$^+$ incorporated per μg of DNA)					
	Untreated		H_2O_2-treated			
Procedure	Mean	SEM	Mean	SEM	Mean difference, counts/min	Index[b]
Manual	469	9	926	97	457	1.97
Robot	378	62	799	13	421	2.11
Manual	312	14	837	61	525	2.68
Robot	273	41	671	48	398	2.46
Manual	544	9	1642	100	1098	3.02
Robot	548	89	1750	48	1202	3.10
Manual	174	29	214[c]	122[c]	40	1.22
Robot	175	1	244[d]	—	49	1.28

[a] Results for four separate ADPRT assays performed on different days and on different patients, compared with the manual procedure. The treated cells received 100 μmol of H_2O_2.
[b] H_2O_2-treated value divided by untreated value.
[c] Mean ± SEM of three tubes; all other results are for duplicate determinations.
[d] Only one result; other tube broken by the robot.

spheres had been detached during an overnight incubation (data not shown).

Discussion

The genotoxic endpoints we used to estimate the influence of antibody-coupled magnetic microspheres attached to the surface of viable T lymphocytes have previously been shown to reflect cell functionality related to cancer risk (*20*).

In analyzing different functions of peripheral blood cells, it is often necessary to first separate the different cell types from one another, to avoid interactions between different cell types, i.e., when the presence of one cell type affects the function of other cell types. Cells may be separated on the basis of their density, size, or surface charge, and the methods chosen, although useful for preparative purposes, produce cell fractions with considerable functional heterogeneity. The development of monoclonal antibodies to cell-surface antigens has provided a tool of unmatched specificity. These antibodies have been used in various affinity-separation methods and are the basis for fluorescence-activated cell sorting. During the last decade the development of magnetic cell-separation methods (*21*), presenting simplicity, swiftness, and the ability to separate large quantities of cells, has attracted an increasing interest.

Blood samples from normal human donors display a normal variation in the amount of cells in each subclass. Such variation is known to influence the result of certain immunological and genotoxic assays (*10, 22*). When magnetic separation technology is used, highly reproducible subsets can be obtained because of the high degree of specificity of the mAbs. Carrying out large numbers of such assays with defined cell subsets would be virtually impossible without magnetic separation technology.

Our purpose here was to develop a simple separation method that would enable us to fractionate viable cell subpopulations according to their surface phenotypes, to perform subsequent assay of cellular variables, and to automate the assay procedures. Development of such a method would enable us to perform assays in the areas of genetic toxicology and immunology on a large-scale basis, as would be required in epidemiological studies or in clinically routine tests.

Genotoxic or immunological functionality tests are not regarded as suitable tools for clinical laboratory testing because of their complexity and high costs, mainly related to the following:

- Cells have a limited survival time; i.e., samples have to be analyzed immediately at arrival to the laboratory.
- The cells have to be handled gently in a well-defined environment.
- All handling of the cells has been manual.

Currently, to demonstrate the content of (e.g.) an enzyme or a hormone in certain cells, one obtains a blood sample, separates and kills the blood cells, harvests the enzyme or hormone, and quantifies the analyte in a test tube. However, this performance does not necessarily reflect the actual biochemical reactions within the cell, because most biochemical reactions depend on pools of precursor substances. Thus it makes a difference if we add a substrate to

Table 5. Proliferative Response of Human MNC to Polyclonal Activators before and after Magnetic Separation of T Cells

	Microspheres present		Microspheres detached	
Activator[a]	[^{3}H]Thd, counts/min per 10^5 cells[b]	% of response of MNC with no added microspheres	[^{3}H]Thd, counts/min per 10^5 cells[b]	% of response of MNC with no added microspheres
PHA, 5 μg	26 ± 4.4	99	27 ± 2.7	103
PHA, 5 μg	25 ± 2.3	96	26 ± 1.6	100
PHA, 5 μg	13 ± 0.8	36	17 ± 1.4	47
SEA, 0.1 ng	37 ± 0.7	131	55 ± 6.2	194
PWM, 1:2500	22 ± 0.7	50	39 ± 1.3	89

[a] PHA, phytohemagglutinin; SEA, staphylococcus enterotoxin A; PWM, pokeweed mitogen. [b] Mean ± SEM, n = 3 each.

(e.g.) a microsomal fraction obtained after rupturing whole cells, or if we add the same substrate to whole cells, where the transportation system of the substrate may be the rate-limiting step. Another example of this is seen in evaluating substances that are carcinogenic/mutagenic. These substances may require oxidative metabolism via the formation of DNA-damaging epoxides before they become active as carcinogens/mutagens. Consequently, different enzymes and co-factors (e.g., NADPH) participate in regulating the quantity of DNA damage induced. Because microsomal enzymes are contained in certain compartments of the cell, where they are membrane bound, it is very important to carry out analyses of such enzymes on viable cells.

Once potentially DNA-damaging epoxides have been produced, they can be inactivated to a non-DNA-damaging metabolite by epoxide hydrolase and GT. The quantities of these enzymes, in relation to each other and in relation to the amount of mixed-function microsomal oxygenases and the concentrations of cofactors such as glutathione, regulate the intracellular balance between DNA-damaging intermediary products. That this intracellular balance can be genetically controlled further emphasizes the importance of the use of viable cells.

At the moment cancer testing or risk estimation methods are extremely rare. The "Pap" test is utilized for cervical cancer and tests for occult blood in the stool are used to assess for cancer in the colon. Assays of carcinoembryonic antigen are used to monitor patients who have been treated for cancer of the colon, breast, or pancreas, and to check for recurrence of cancer in the lung. However, the assay of carcinoembryonic antigen has not been useful in screening for malignancy because of the high false-positive and false-negative percentages in normal populations (*23*).

Given the possibility of a genetic link in certain types of cancer, it is important to test the close relatives of affected patients for cancer susceptibility. As for the major organ sites of cancer, e.g., breast, colon, and lung, a considerable portion of the population should profit by an early cancer detection—if such tests could be performed on a large-scale basis at a reasonable cost.

However, it is not sufficient simply to measure the degree of cancer susceptibility. This piece of information must come with the benefit to the patients. Some of the tests described above can be used to monitor altered susceptibility to cancer after changing health-related behaviors, i.e., quitting smoking, decreasing alcohol intake, or stopping working in an unhealthy environment.

Testing for carcinogenicity is another field requiring automated analysis of viable cells. At present, some available tests can determine responses to genotoxic agents, e.g., automated toxicity testing of mutated bacterial cells. However, so far, tests involving human cells are strictly manual (*1*). As described above, the use of magnetic microspheres attached to the surface of viable cells makes possible the performance of complicated and lengthy assays through the use of laboratory robotics. Thus, the rat hepatocyte test and the HeLa cell test for DNA damage (e.g.) could potentially be adopted to automation with use of the techniques presented here.

Similarly, the pharmaceutical industry has high demands regarding the testing of new compounds produced in their laboratories for carcinogenicity and toxicity. Thus, reliable testing for mutagenicity and carcinogenicity on a relevant cell system early in the development of a drug, and at a reasonable cost, would be advantageous.

In conclusion, we used magnetically responsive microspheres bearing surface-immobilized monoclonal antibodies against cell-surface antigens to obtain a subset of cells for further genotoxicological and immunological assays. We were surprised to find how little the attached magnetic microspheres interfered with the assays performed. We believe this technique will be useful in assays for biochemical variables in such fields as genotoxicology, cancer susceptibility, risk estimation, and environmental toxicology. This technique also opens up the possibility of performing functional immunological assays routinely at a reasonable cost.

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